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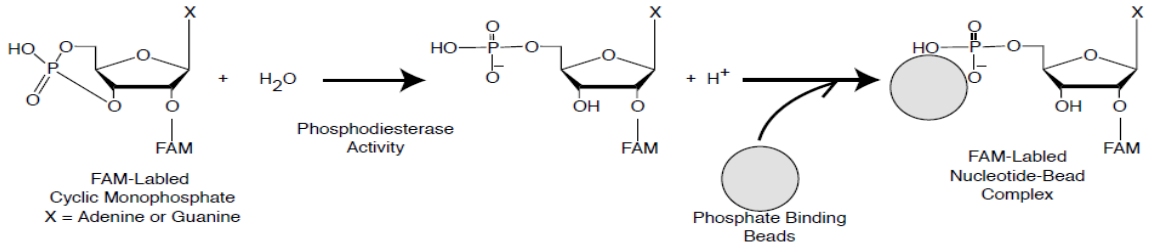
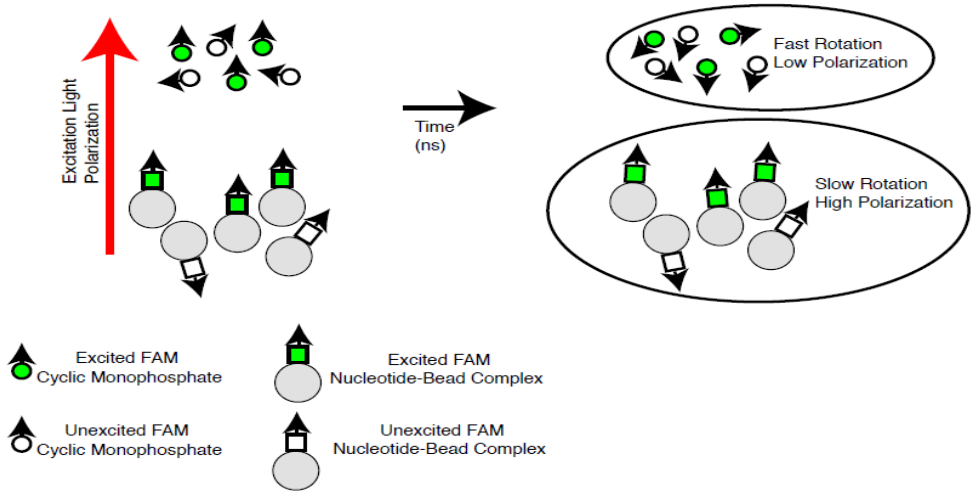
Data Sheet
PDE7A Assay Kit
Catalog # 60373

BACKGROUND: Phosphodiesterases (PDEs) play an important role in the dynamic regulation of cAMP and cAMP signaling. PDE7A is widely expressed in various tissues including skeletal muscle, T lymphocytes, brain and pancreas and plays and an important role in the regulation of osteoblastic differentiation.

DESCRIPTION: The PDE7A Assay Kit is designed for identification of PDE7A inhibitors using fluorescence polarization. The assay is based on the binding of a fluorescent nucleotide monophosphate generated by PDE7A to the binding agent. PDE7A catalyzes the hydrolysis of the phosphodiester bond in dye-labeled cyclic adenosine monophosphate (cAMP). Nanoparticle beads selectively bind the phosphate group in the nucleotide product. This increases the size of the nucleotide relative to unreacted cAMP. Since the degree of polarization of a fluorophore is inversely related to its molecular rotation, dyes attached to the slowly-rotating nucleotide-bead complexes will not have time to reorient and therefore will emit highly polarized light. Conversely, dyes attached to the rapidly-rotating cyclic monophosphates will obtain random orientations and emit light with low polarization.





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The PDE7A inhibitor screening assay kit comes in a convenient 384-well format, including purified PDE7A enzyme, fluorescently labeled PDE7A substrate (cAMP), binding agent, and PDE assay buffer for 384 enzyme reactions. The key to the *PDE7A Assay Kit* is the specific binding agent. Using this kit, only two simple steps on a microtiter plate are required for PDE7A reactions. First, the fluorescently labeled cAMP is incubated with a sample containing PDE7A for 1 hour. Second, a binding agent is added to the reaction mix to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization.*

COMPONENTS:

Catalog #	Component	Amount	Storage	
60070	PDE7A recombinant enzyme	1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
60200	FAM-Cyclic-3′, 5′-AMP: 20 µM	50 µl	-80°C	
60393	PDE assay buffer	25 ml	-20°C	
60390	Binding Agent	250 µl	+4°C	
60391	Binding Agent Diluent (cAMP)	25 ml	+4°C	
	Black, low binding, 384 microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring fluorescence polarization

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: 6 months from date of receipt when stored as directed.

REFERENCE(S):
1. Malik, R. et al. (2008) Appl. Microbiol. Biotechnol.77 (5): 1167-1173.
2. Pekkinen, M. et al. (2008) Bone. 43 (1): 84-91.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:
1) Dilute 20 µM **FAM-Cyclic-3',5'-AMP** substrate stock solution 100-fold with **PDE Assay Buffer** to make a 200 nM solution. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

2) Add 12.5 µl of diluted **FAM-Cyclic-3',5'-AMP** (200 nM) to each well designated "Substrate Control", "Positive Control", and "Test Inhibitor". Add 12.5 µl of **PDE Assay Buffer** to each well designated "Blank".

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3) Add 2.5 µl of inhibitor solution to each well designated “Test Inhibitor”. Add 2.5 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Blank”, “Substrate Control” and “Positive Control”.

4) Add 10 µl of **PDE Assay Buffer** to the wells designated as the "Blank" and "Substrate Control”.

5) Thaw **PDE7A** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot **PDE7A** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PDE7A** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **PDE7A** in **PDE Assay Buffer** to 8 pg/µl (80 pg/reaction) in **PDE Assay Buffer***. Initiate reaction by adding 10 µl of diluted **PDE7A** to the wells designated for the “Positive Control” and “Test Inhibitor”. Discard any remaining diluted enzyme after use. **Note: optimal enzyme concentration may vary with the specific activity of the enzyme*.

7) Incubate at room temperature for 1 hour.

	Blank	Substrate Control	Positive Control	Test Inhibitor
FAM-Cyclic-3’,5’-AMP (200 nM)	–	12.5 µl	12.5 µl	12.5 µl
PDE assay buffer	22.5 µl	10 µl	–	–
Test Inhibitor	–	–	–	2.5 µl
Inhibitor Buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
PDE7A (8 pg/µl)	–	–	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Step 2:

1) Shake the tube containing the **Binding Agent** to ensure it is thoroughly mixed. Mix **Binding Agent** thoroughly and dilute **Binding Agent** 1:100 with the **cAMP Binding Agent Diluent .**

2) Add 50 µl diluted **Binding Agent** to each well. Incubate at room temperature for 20 minutes with slow shaking.

3) Read the fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 485 ± 5 nm and detection of emitted light ranging from 528 ± 10 nm. Blank value is subtracted from all other values**.**

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

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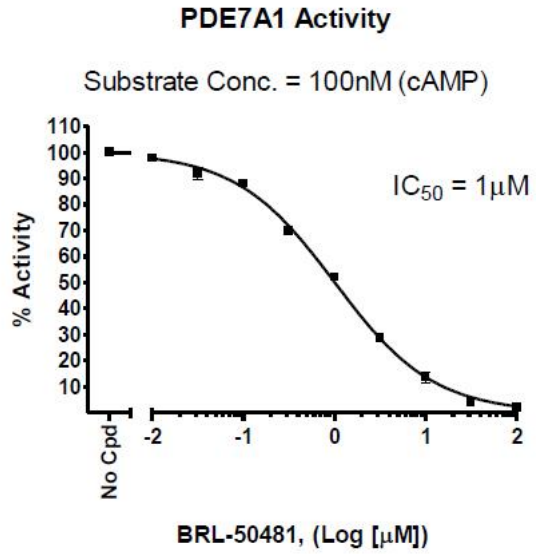
EXAMPLE OF ASSAY RESULTS:



Inhibition of PDE7A by BRL-50481, measured using the *PDE7A Assay Kit*, BPS Bioscience # 60373. Fluorescence polarization was measured at 528 nm using a Tecan M-1000 fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS :

Product	Catalog #	Size
PDE7A	60070	10 µg
PDE7B	60071	10 µg
Mouse PDE7A	60072	10 µg
Mouse PDE7A	60073	10 µg
Rat PDE7A	60074	10 µg
Rat PDE7A	60075	10 µg
PDE7A Assay Kit	60370	96 rxns.
PDE7B Assay Kit	60371	96 rxns.
PDE Assay Kit	60300	96 rxns.
PDE1A Assay Kit	60310	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE10A Assay Kit	60400	96 rxns.
FAM-cAMP Substrate	60201	100 nmole.
PDE7A	60070	10 µg
PDE7B	60071	10 µg
Mouse PDE7A	60072	10 µg
Mouse PDE7A	60073	10 µg
Rat PDE7A	60074	10 µg
Rat PDE7A	60075	10 µg
PDE7A Assay Kit	60370	96 rxns.
PDE7B Assay Kit	60371	96 rxns.
PDE Assay Kit	60300	96 rxns.
PDE1A Assay Kit	60310	96 rxns.
PDE3A Assay Kit	60330	96 rxns.
PDE4A Assay Kit	60340	96 rxns.
PDE5A Assay Kit	60350	96 rxns.
FAM-cAMP Substrate	60200	100 nmole